APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2000, p. 2682–2684 Vol. 66, No. 6
0099-2240/00/$04.00+0

Fermentation of Fructooligosaccharides by Lactic Acid Bacteria and Bifidobacteria†

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Received 17 December 1999/Accepted 21 March 2000

Lactic acid bacteria and bifidobacteria were screened of their ability to ferment fructooligosaccharides (FOS) on MRS agar. Of 28 strains of lactic acid bacteria and bifidobacteria examined, 12 of 16 *Lactobacillus* strains and 7 of 8 *Bifidobacterium* strains fermented FOS. Only strains that gave a positive reaction by the agar method reached high cell densities in broth containing FOS.

The number of food and other dietary products containing live *Bifidobacterium* and *Lactobacillus* bacteria has increased significantly in recent years (20); this is due, in part, to the beneficial effects these probiotic organisms are believed to confer. Although substantial research efforts are currently in progress to investigate these claims, the available evidence indicates that ingestion of probiotic bacteria may promote desirable changes in the gastrointestinal tracts of humans and other animals (8, 12, 13, 16). For example, ingestion of probiotic bacteria may reduce the severity and frequency of diarrheal diseases, as well as improve lactose digestibility among lactose-intolerant individuals (6, 18).

The criteria established to select suitable *Bifidobacterium* and *Lactobacillus* strains and to identify which strains possess these desired traits are also the subject of much research interest. Several specific characteristics possessed by these bacteria are thought to be desirable or necessary (7). Probiotic bacteria should resist low pH and survive gastric acidity, they should tolerate bile salts at concentrations present in the intestinal tract, they should adhere to intestinal mucosal cells, and they should provide clinically proven benefits. Recently, it has been suggested that the ability of probiotic bacteria to ferment oligosaccharides may be an especially important characteristic (1, 2, 4, 9, 14, 21). This is because the availability of carbohydrates that escape metabolism and adsorption in the small intestine have a major influence on the microflora that become established in the colon. If certain carbohydrates, such as oligosaccharides, are fermented only by specific strains of bifidobacteria and lactobacilli, then diets containing so-called "prebiotic" substrates could select for those strains of probiotic bacteria. Such a scenario, if true, could be "one of the most important stories to emerge in nutrition and gut microbiology since the turn of the century" (9).

One specific group of oligosaccharides that has attracted much commercial interest as prebiotics is the fructooligosaccharides (FOS). These compounds, which are marketed commercially as Raftilose and Nutraflora, can be obtained from natural sources (e.g., inulin) or synthesized naturally from sucrose (2, 5, 15, 23). The FOS product Nutraflora belongs to the latter category. It consists of a glucose monomer (G) linked α-1,2 to two or more β-2,1-linked fructosyl units (F), to give 1-kestose (GF_2), nystose (GF_3), and 1^F-fructofuranosyl nystose (GF_4). FOS have been self-affirmed by the manufacturers as GRAS (generally recognized as safe) (19) and have been added to infant formulas, yogurt, and other food products and food supplements.

Despite the considerable commercial and research interests in oligosaccharides and probiotic bacteria, relatively little is known about which strains actually metabolize these materials. Because commercial oligosaccharide preparations often contain glucose, fructose, sucrose, or other fermentable sugars, it has been difficult to establish that growth in FOS-containing medium is due to actual utilization of FOS (10, 11, 22). In this study, we incorporated a pure form of FOS into MRS medium in order to identify lactic acid bacteria and bifidobacteria capable of fermenting FOS.

A commercial FOS mixture containing three FOS species, GF_2 (32.0%), GF_3 (53.6%), and GF_4 (9.8%), was supplied by The GTC Nutrition Company (Westminster, Colo.). The balance consisted of 2.3% glucose and fructose and 2.3% sucrose. To prepare pure FOS, a 40% (wt/vol) FOS solution was applied to a column (30 by 5 cm) containing activated charcoal (Sigma Chemical Co., St. Louis, Mo.). Glucose and fructose were eluted by using distilled water, and sucrose was eluted by the addition of 5% ethanol. Finally, FOS was eluted by using 15% ethanol, and the solution was lyophilized (FTS Systems, Inc., Stone Ridge, N.Y.). The purity of the FOS and the concentration of each FOS moiety (GF_2, GF_3, and GF_4) were determined by high-pressure liquid chromatography (HPLC) (Waters Corp., Milford, Mass.) using an Aminex HPX-42C column (0.78 by 30 cm; Bio-Rad Laboratories, Hercules, Calif.) and an RI-410 detector (Waters). The column temperature was kept constant at 85°C, and water was used as the mobile phase at a flow rate of 0.6 ml/min.

An MRS-FOS agar medium was prepared by adding 2% (wt/vol) purified FOS to MRS agar (3) containing 0.05% L-cysteine, 1.5% agar, and 30 mg of bromcresol purple per liter. The MRS basal medium (i.e., MRS without carbohydrate) was autoclaved, and the FOS was filter sterilized and then added to the tempered agar. A total of 28 strains were evaluated, including 6 strains used as commercial probiotics (Table 1). Each strain was initially grown in MRS broth and then diluted and spread onto the MRS-FOS agar plates to give approximately 25 to 50 colonies. The plates were incubated anaerobically for 24 h. Strains that fermented FOS (and produced acid end products) grew as colonies surrounded by a yellow zone (>3 mm) against a purple background. Nonfermenting colonies produced smaller white colonies without a yellow zone. Thus, although the basal MRS medium was sufficiently rich to pro-

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† Published as paper no. 12877, Journal Series Nebraska Agricultural Experiment Station, Lincoln.

TABLE 1. FOS utilization by lactic acid bacteria and bifidobacteria

Organism	Source[a]	Growth on MRS-FOS[b]	OD[c]	pH[d]
Lactobacillus bulgaricus B734	NRRL	−	0.5	6.3
Lactobacillus bulgaricus CR5	USU	−	0.5	6.4
Lactobacillus bulgaricus CR14	USU	+	2.6	4.5
Lactobacillus acidophilus 33200	ATCC	+	1.4	4.5
Lactobacillus acidophilus 837	ATCC	+	1.8	4.1
Lactobacillus acidophilus DDS-1	NC	+	1.5	4.4
Lactobacillus acidophilus NCFM	NCSU	+	1.6	4.3
Lactobacillus plantarum 4008	ATCC	+	2.8	4.5
Lactobacillus plantarum 1195	NRRL	+	1.9	4.0
Lactobacillus plantarum 12006	IFO	+	2.4	4.4
Lactobacillus plantarum MR240	Rhodia	+	2.4	4.6
Lactobacillus lactis 448	USU	−	0.8	5.6
Lactobacillus casei 685	UNL	+	1.9	4.9
Lactobacillus casei MR191	Rhodia	+	2.4	4.0
Lactobacillus strain GG	ATCC	−	0.5	5.9
Streptococcus thermophilus 19987	ATCC	−	0.6	5.4
Streptococcus thermophilus 14485	ATCC	−	0.7	5.7
Streptococcus thermophilus 19258	ATCC	−	0.6	5.5
Streptococcus thermophilus MTC321	Rhodia	−	0.7	5.9
Bifidobacterium adolescentis 15705	ATCC	+	1.3	4.6
Bifidobacterium adolescentis 15706	ATCC	+	1.3	4.5
Bifidobacterium breve 15698	ATCC	+	1.8	4.4
Bifidobacterium breve 15700	ATCC	+	1.6	4.6
Bifidobacterium bifidum 15696	ATCC	−	0.8	5.0
Bifidobacterium infantis 17930	ATCC	+	1.7	4.4
Bifidobacterium infantis 25962	ATCC	+	1.8	4.4
Bifidobacterium longum 15708	ATCC	+	1.4	4.6

[a] NRRL, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill.; USU, Utah State University, Logan, Utah; ATCC, American Type Culture Collection, Rockville, Md.; NC, Nebraska Cultures, Lincoln, Nebr.; NCSU, North Carolina State University, Raleigh, N.C.; IFO, Institute for Fermentation, Osaka, Japan; Rhodia, Rhodia Dairy Ingredients, Madison, Wis.; UNL, University of Nebraska—Lincoln Culture Collection, Lincoln.

[b] Growth on MRS-FOS agar was positive (+) if colonies were surrounded by a yellow zone and negative (−) if no yellow zone was apparent.

[c] Optical density (OD) at 625 nm after 24 h in MRS-FOS broth.

[d] pH after 24 h in MRS-FOS broth.

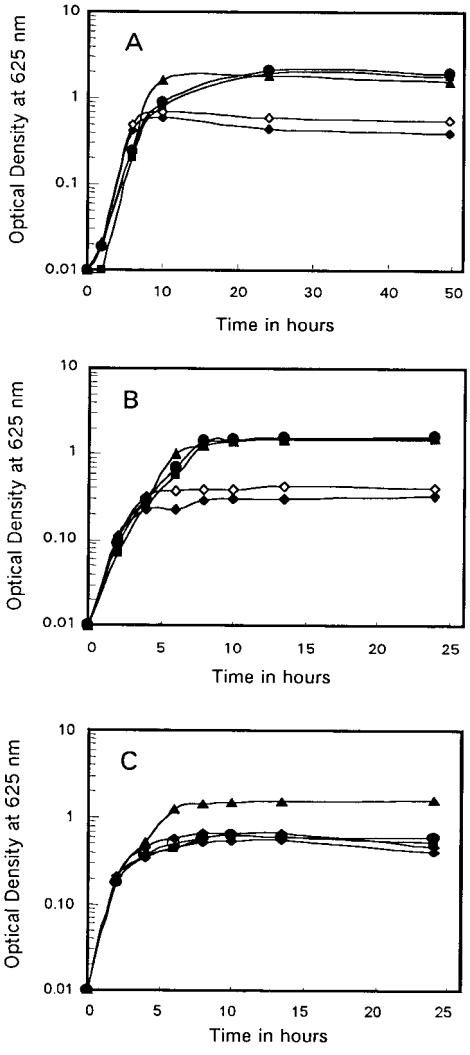

FIG. 1. Growth of *B. infantis* 17930 (A), *L. acidophilus* NCFM (B), and *Lactobacillus* strain GG (C) in MRS broth containing either no added sugar (◆), 2% glucose (▲), 2% commercial FOS (●), or 2% pure FOS (■). To account for the glucose, fructose, and sucrose in the commercial FOS, cells were also grown in MRS broth containing equivalent amounts of these sugars (◇).

mote colony formation by all of the 28 strains examined, only the 19 strains that could ferment FOS produced enough acid to cause a noticeable color change (Table 1). All of the *Lactobacillus acidophilus* strains fermented FOS, a result consistent with that recently reported by Sghir et al. (17). Among the FOS-fermenting *L. acidophilus* isolates were two strains, DDS-1 and NCFM, that are widely promoted as probiotics. Three other commercial probiotic strains, *L. plantarum* MR240, *L. casei* MR191, and *L. casei* 685, also fermented FOS, whereas *Lactobacillus* strain GG, one of the best-studied probiotic strains, was found to be a nonfermenter. Interestingly, most of the *L. bulgaricus* and *Streptococcus thermophilus* strains, ordinarily used for yogurt manufacture, were FOS nonfermenters.

To confirm that growth was dependent on FOS utilization, strains were also inoculated into MRS basal broth containing either 2% glucose, 2% commercial FOS or 2% purified FOS as the carbohydrate source. To account for the glucose, fructose, and sucrose in the commercial FOS, an equivalent amount of these sugars was added to a set of control MRS broths to give a final carbohydrate concentration of 0.1%. All strains, even those previously identified as FOS nonfermenters, grew in MRS-FOS broth, due apparently to the presence of background levels of carbohydrate in the basal MRS broth or, to a limited extent, to contaminating sugars in the commercial FOS (Table 1 and Fig. 1). However, final cell densities of FOS-fermenting *Bifidobacterium infantis* 17930 (Fig. 1A) and *L. acidophilus* NCFM (Fig. 1B) were twice that of the FOS-nonfermenting *Lactobacillus* strain GG (Fig. 1C). We also observed that when *L. acidophilus* NCFM was first grown in MRS broth (containing 2% glucose) and then transferred to MRS-FOS broth, only a short lag phase occurred. In addition, growth rates on FOS and glucose were the same. These results suggest that FOS utilization did not require an induction period and that FOS was equally as good a substrate as glucose in supporting growth.

That only FOS-fermenting strains consumed FOS was also confirmed more directly by HPLC analysis of the fermentation broths (Fig. 2). In these experiments, cultures were grown in MRS-FOS broth containing 25 mM concentrations each of GF_2 and GF_3 and 8 mM GF_4. *L. plantarum* 1195, a FOS fermenter, rapidly consumed GF_2 and GF_3 at near equal rates and reduced the pH to below 4.0. Utilization of these saccharides by *Lactobacillus* strain GG was minimal, and the pH decreased to only 6.0. Interestingly, none of the strains exam-

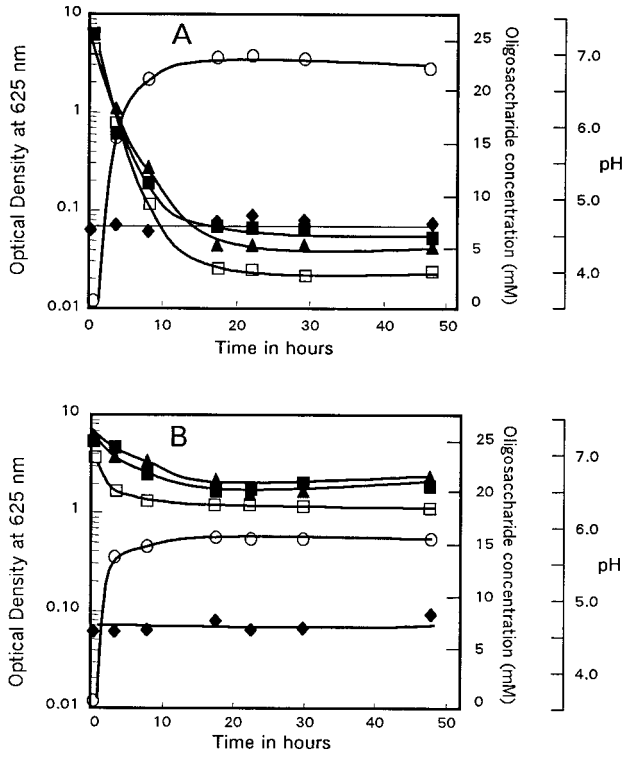

FIG. 2. Utilization of FOS by *L. plantarum* 1195 (A) and *Lactobacillus* strain GG (B). Cells were grown in MRS-FOS broth containing 25 mM GF_2 (■), 25 mM GF_3 (▲), or 8 mM GF_4 (◆). The optical density (○) and pH (□) are also shown.

ined were able to use the GF_4 moiety. We are currently studying the molecular basis for FOS metabolism by these strains.

This research was supported, in part, by a grant from Dairy Management, Inc.

We thank The GTC Nutrition Company for providing the FOS. We also thank Lisa Durso for reviewing the manuscript.

REFERENCES

1. **Collins, M. D., and G. R. Gibson.** 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am. J. Clin. Nutr. **69**(Suppl.)**:**1052S–1057S.
2. **Crittenden, R. G.** 1999. Prebiotics, p. 141–156. *In* G. W. Tannock (ed.), Probiotics: a critical review. Horizon Scientific Press, Wymondham, Norfolk, United Kingdom.
3. **de Man, J. C., M. Rogosa, and M. E. Sharpe.** 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. **23:**130–135.
4. **Gibson, G. R., and M. B. Roberfroid.** 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. **125:**1401–1412.
5. **Hikada, H., M. Hirayama, and N. Sumi.** 1988. A β-FOS-producing enzyme from *Aspergillus niger* ATCC 20611. Agric. Biol. Chem. **52:**1181–1187.
6. **Kolars, J. C., M. D. Levin, M. Aouiji, and D. A. Savaiano.** 1984. Yogurt—an autodigesting source of lactose. N. Engl. J. Med. **310:**1–3.
7. **Kullen, M. J., and T. R. Klaenhammer.** 1999. Genetic modification of lactobacilli and bifidobacteria, p. 65–84. *In* G. W. Tannock (ed.), Probiotics: a critical review. Horizon Scientific Press, Wymondham, Norfolk, United Kingdom.
8. **Kurmann, J. A., and J. L. Rašić.** 1991. The health potential of products containing bifidobacteria, p. 117–158. *In* R. K. Robinson (ed.), Therapeutic properties of fermented milks. Elsevier Applied Science Publishers, Ltd., London, England.
9. **Macfarlane, G. T., and J. H. Cummings.** 1999. Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? Br. Med. J. **318:**999–1003.
10. **McKellar, R. C., and H. W. Modler.** 1989. Metabolism of fructo-oligosaccharides by *Bifidobacterium* spp. Appl. Microbiol. Biotechnol. **31:**537–541.
11. **Modler, H. W., R. C. McKellar, and M. Yaguchi.** 1990. Bifidobacteria and bifidogenic factors. Can. Inst. Food Sci. Technol. J. **23:**29–41.
12. **Ouwehand, A. C., P. V. Kirjavainen, C. Shortt, and S. Salminen.** 1999. Probiotics: mechanisms and established effects. Int. Dairy J. **9:**43–52.
13. **Reid, G.** 1999. The scientific basis for probiotic strains of *Lactobacillus*. Appl. Environ. Microbiol. **65:**3763–3766.
14. **Roberfroid, M. B.** 1998. Prebiotics and synbiotics: concepts and nutritional properties. Br. J. Nutr. **80**(Suppl. 2)**:**S197–S202.
15. **Roberfroid, M. B., J. A. E. Van Loo, and G. R. Gibson.** 1998. The bifidogenic nature of chicory inulin and its hydrolysis products. J. Nutr. **128:**11–19.
16. **Sanders, M. E.** 1993. Summary of conclusions from a consensus panel of experts on health attributes of lactic cultures: significance to fluid milk products containing cultures. J. Dairy Sci. **76:**1819–1828.
17. **Sghir, A., J. M. Chow, and R. I. Mackie,** 1998. Continuous culture selection of bifidobacteria and lactobacilli from human faecal samples using fructo-oligosaccharide as selective substrate. J. Appl. Microbiol. **85:**769–777.
18. **Siitonen, S., H. Vapaatalo, S. Salminen, A. Gordin, M. Saxelin, R. Wikberg, and A.-L. Kirkkola.** 1990. Effect of *Lactobacillus* GG yoghurt in prevention of antibiotic associated diarrhoea. Ann. Med. **22:**57–59.
19. **Speigel, J. E., R. Rose, P. Karabell, V. H. Frankos, and D. F. Schmitt.** 1994. Safety and benefits of fructooligosaccharides as food ingredients. Food Technol. **48:**85–89.
20. **Tannock, G. W.** 1999. Introduction, p. 1–4. *In* G. W. Tannock (ed.), Probiotics: a critical review. Horizon Scientific Press, Wymondham, Norfolk, United Kingdom.
21. **Wang, X., and G. R. Gibson.** 1993. Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J. Appl. Bacteriol. **75:**373–380.
22. **Yazawa, K., K. Imai, and Z. Tamura.** 1978. Oligosaccharides and polysaccharides specifically utilizable by bifidobacteria. Chem. Pharm. Bull. **26:** 3306–3311.
23. **Yun, J. W., M. G. Lee, and S. K. Song.** 1994. Batch production of high-content fructo-oligosaccharides from sucrose by the mixed-enzyme system of fructofuranosidase and glucose oxidase. J. Ferment. Bioeng. **77:**159–163.